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Research Article

Role of seed priming in improving seed germination and seedling growth of maize (*Zea mays* L.) under rain fed condition

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ABSTRACT

Seed priming enhances early seed emergence and proper crop stand establishment which enables the crop to capture more soil moisture, nutrients, and solar radiation. An on-farm experiment was conducted in Okhaldhunga, Nepal to study the effect of six different priming treatments (50 millimoles common salt solution, 200 millimoles urea solution, 1 percent MOP solution, 2.5 times diluted urine, water, and control) on germination and growth of two maize varieties, Manakamana-3 and Nutan-IL60. The experiment was set up in factorial randomized complete block design with three replications. Various germination traits: germination percent, seedling vigour, germination index, mean germination time, and seedling growth traits: shoot length, root length, and dry root weight, were studied. Nitrogen-based (Urea and Urine) priming resulted the most desirable change for the studied traits. Nitrogen-based priming also had significant positive effect on stress tolerance related traits like root length and dry root weight.

Keywords: Germination index, nitrogen-based priming, seed priming, urea, urine

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INTRODUCTION

Maize (*Zea mays* L.) is second major crop after rice in term of area and production in Nepal (MOAD, 2017). Nepal has the highest per capita maize consumption (98 g/person/day) in the South Asia (Ranum *et al.*, 2014). The farm level yield of maize (2.55 t/ha) is not satisfactory as compared to attainable yield (5.7 t/ha) in Nepal (MOAD, 2017; Karki *et al.*, 2015).There is a huge gap between the demand and production level of Maize in Nepal affected by various technological and socio-economic factors. Among the many factors of production, seed is the principle factor that greatly affects the production and productivity of crop (Shrestha & Shrestha, 2017). Large numbers of farmers in Nepal are still far from the reach of quality seeds (Bhandari *et al.*, 2017). Poor seed germination, drought stress and *Turcicum* leaf blight are the major maize yield limiting factors at the eastern hills of Nepal (Tiwari *et al*., 2001). Seasonal drought and poor seed quality further constrains the maize productivity. At field condition, uniform plant establishment and vigorous early growth are a pre-requisite for a successful crop production which is compromised under different abiotic stresses like drought. In such cases, seed priming, soaking seeds in water for a certain amount of time followed by surface drying, could help to gain rapid germination, better establishment and increasing crop yield under unfavourable environmental conditions (Mousavi *et al.*, 2012).

Priming helps to enhance the root system of maize plants. Healthy plants with well-developed root systems can withstand adverse conditions better than those interrupted at an early stage (Pegah *et al*., 2008). The problem of poor germination can be combated by seed priming which in turn gives proper crop stand and increased yield (Khan, 1992). Seed priming increases the rate of hydration which helps to combat the drought stress during germination, emergence and growth of maize varieties (Ajirloo *et al.*, 2013; Tian *et al.,* 2014). It stimulates the metabolic processes involved in early phases of germination, thereby aiding the primed seeds emerge faster, grow more vigorously, and perform better in adverse conditions. During priming, the damaged or pest infested floating seeds are discarded which also helps to reduce the problem of poor-quality seed.

According to Koirala (2017), faster emergence, better and more uniform stands, less need to re-sow, more vigorous plants, earlier flowering, earlier harvest and higher and stable grain yield were the benefits of seed priming in maize. Priming treatment shortened silking and maturity days in maize with an increase in grain yield by 11.6% and maximum yield increase by 27.8% in Manakamana-1 at Palpa, Gulmi and Myagdi districts of Nepal (Koirala *et al*., 2006). Majority of farmers (75.1 to 88.9%) participated in Mother-Baby trial conducted at western Nepal, were keen to adopt seed priming technology in maize (Koirala, 2017).

Farmers needs locally available priming reagents that are both economic and user friendly, to overcome the problem of low seedling establishment in Nepal. Hence, this experiment was undertaken at farmers' field to identify the effects of various priming treatments in seed germination and seedling establishment of maize under eastern hills condtion of Nepal.

MATERIALS AND METHODS

A field experiment was laid out in randomized complete block design with three replications at farmers' field condition at Okhaldhunga, Nepal during 2018. The soil from the research field was slightly alkaline with pH of 7.6 unlike most soils around eastern Nepal which are acidic (Bajracharya *et al.*, 2007) with sandy loam texture. The research site (27.3065° N,

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86.5098° E) is located at an elevation of 1700 masl having sub-tropical to temperate climate with an average annual rainfall of 1793 mm and average annual temperature of 17.3°C (Figure 1).

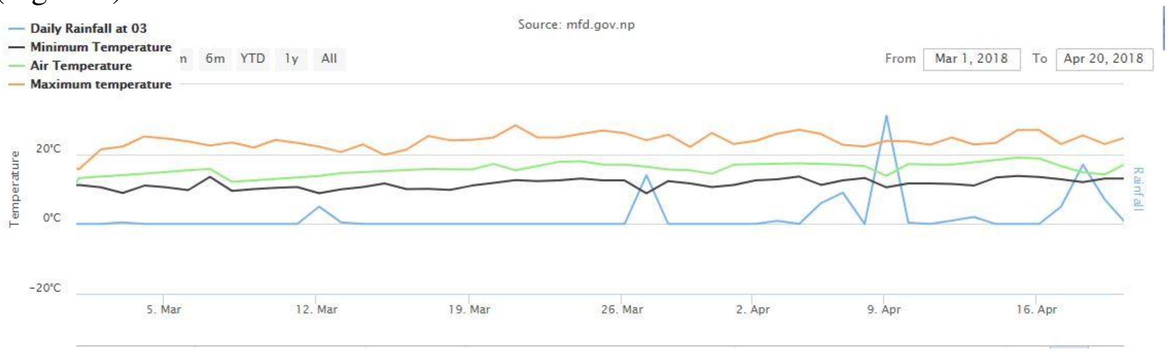

Figure 1: Agro-meteorological data of the research site (Source: www.mfda.gov.np)

Table 1: Pedigree, origin and source of germplasm

S.N.	Variety	Pedigree	Origin	Source
1	Manakamana 3	BA 93	CIMMYT	NARC, Khumaltar
2	Nutan IL 60	-	India	Everest Agrovet, Lalitpur

Two maize varieties (Factor A): Manakamana-3 (an open pollinated variety) and Nutan-IL60 (a commercial hybrid variety), were treated with six hydro-priming reagents (Factor B): no priming, water, 50 mM common salt (200 mL solution), 200 mM urea, urine (2.5 times dilution) and 1% Muriate of Potash (MOP) (Table 1), to evaluate germination and seedling growth/establishment. Chemical equations were used to calculate mentioned concentrations of 200 mL working solution which resulted in 0.6 g salt, 1.2 g urea, 2 g MOP and 80 mL urine. Seeds were primed for 12 hours followed by 12 hours air drying.

Table 2: Treatments used in the experiment

Factor A	Factor B	Treatment Combinations
Nutan IL60 (V1)	Control (C1)	V1C1: Nutan IL60 without priming
	Urea (C2)	V1C2: Nutan IL60primed with urea
	Salt (C3)	V1C3: Nutan IL60 primed with salt
	Urine (C4)	V1C4: Nutan IL60 primed with urine
	MOP (C5)	V1C5: Nutan IL60 primed with MOP
	Water (C6)	V1C6: Nutan IL60 primed with water
Manakamana-3 (V2)	Control (C1)	V2C1: Manakamana-3 without priming
	Urea (C2)	V2C2: Manakamana-3 primed with urea
	Salt (C3)	V2C3: Manakamana-3 primed with salt
	Urine (C4)	V2C4: Manakamana-3 primed with urine
	MOP (C5)	V2C5: Manakamana-3 primed with MOP
	Water (C6)	V2C6: Manakamana-3 primed with water

Seeds of each variety was sown in three rows accommodating 20 hills per row with the plot area of 7.5 m^2 and 75 x 25 cm spacing. Germination percentage (GP), germination index (GI), vigour index (VI) and mean germination time (MGT) were calculated according to Moradi *et al.* (2012) as follows:

- $$Germination\ Percentage\ (GP) = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds\ sown} x100$$

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- $Germination\ Index\ (GI) = \frac{Number\ of\ germinated\ seeds}{Days\ of\ first\ count} + \ldots\ldots + \frac{Number\ of\ germinated\ seeds}{Days\ of\ last\ count}$
- $Vigor\ Index\ (VI) = \frac{Germination\ percentage}{Seedling\ length\ (cm)}\ x\ 100$

  VI was calculated 30 days after sowing
- $Mean\ Germination\ Time\ (MGT) = \frac{\Sigma TiNi}{\Sigma Ni}$

  Where, T_i = Total time for germination (in days)

  N_i = Number of seedlings emerged

Other observations taken during the experiment were:

- Root length (cm): Ten seedlings were randomly selected in each plot on the eighth day after first seed germination. The root length was measured from the tip of the primary root to base of hypocotyls with the help of a scale and mean root length was expressed in centimetres.
- Shoot length (cm): Shoot length was recorded from the same seedling used for measuring root length. The shoot length was measured from the tip of the primary leaf to the base of the hypocotyls and mean shoot length was expressed in centimetres.
- Root dry weight (g): The ten seedlings used for root and shoot length measurements were put in butter paper packet and dried in hot air oven at 70 ± 10^0C for 24 hours. The mean dry weight of the seedling was recorded and expressed in grams per plant.

Statistical analysis: Generated data were entered in excel and subjected to analysis of variance (ANOVA) using R-Studio version 3.5.0 with software package Agricolae. Graphs and bar diagrams were prepared using MS-Excel. Duncan's multiple range test (DMRT) was performed to compare treatment means. The significant differences between genotypes were determined using least significant difference (LSD) test at probability level of 0.01 or 0.05 where the effects of the treatments were significant at 1% or 5% level of probability, respectively (Gomez & Gomez, 1984; Sharma *et al.*, 2016; Shrestha, 2019; Kandel & Shrestha, 2019).

RESULT AND DISCUSSION

The response of two maize varieties for different seed treatments was interpreted in terms of germination and growth parameters. Both variety and priming treatments had significant effects on the studied parameters (Table 2). Moreover, the interaction of variety and priming with nitrogen-based treatment; both organic and inorganic (Urea and Urine) had a significant effect on all parameters tested at 5% significant level (Table 2). Priming with Urea and Urine improved the germination parameters (TG, MGT) and growth parameters (RL, SL, RDW and VI) in both genotypes, as compared to Salt, MOP, hydro-primed and un-primed seeds (Table 3).

Table 3: ANOVA for germination and growth parameters of two maize varieties

Source of variation	FGP	GI	MGT	VI	RL	SL	RDW
Variety	78.47*	12754***	94.64***	129.16***	937.4***	176.71***	0.01914**
Priming	284.04	3785***	3.77***	164.89***	34.6***	149.31***	0.7334***
Priming × Variety	53.68	991***	1.96***	32.07**	7.0***	41.38***	0.01050**
Error	93.49	10	0.15	7.94	0.9	3.53	0.00205

FGP: Final germination percent; GI: Germination index; MGT: Mean Germination time; VI: Vigour index; RL: Root Length; SL: Shoot Length; RDW: Root Dry Weight

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Germination Parameters

Final germination percent (FGP) and germination index (GI)
Results revealed that all the seed priming treatments improved the FGP in Manakamana-3 while MOP and salt treatments impeded germination in Nutan-IL60 (Table 3). In comparison to control, the highest germination percent and germination index was observed under nitrogen based priming treatments in both varieties. In Manakamana-3, highest FGP (89%) and GI (178) was observed in urea based priming for 12h. While, in Nutan-IL60, FGP was highest under 24h urea based priming (88%) and GI was maximum under 24h urine based priming (171) (Table 3).

Mean germination time (MGT)
All seed priming treatments caused a significant reduction in MGT (Table 3). Un-primed seed (control) took the longest time to germinate (12 and 15 days) in both varieties. Seed subjected to nitrogen based priming with urea and urine had reduced MGT by 2-4 days as compared to control (un-primed) and other treatments (Table 3). The probable reason for the early emergence of the primed seeds may be the completion of pre-germination metabolic activities that makes the seed ready for radicle protrusion. These results are in coherence with the findings of Ahammad *et al.* (2014). Priming reagents significantly affects the amount of total reducing sugars in seeds by influencing the activity of alpha amylase, which in turn hastens germination and reduces MGT in primed seeds (Afzal *et al.*, 2008).

Table 4: Germination parameters for two maize varieties under different priming treatments at Okhaldhunga, Nepal

Treatment	Final Germination Percentage		Germination Index		Mean Germination Time (Days)	
	Manakamana-3	Nutan-IL60	Manakamana-3	Nutan-IL60	Manakamana-3	Nutan-IL60
Control	75.0	72.9	121.3	90.0	12	15
MOP-12	75.0	70.8	134.7	98.3	10	15
Salt-12	76.0	63.5	155.3	87.0	10	15
Urea-12	88.5	85.4	178.0	155.3	10	12
Urine-12	80.2	86.5	157.0	`159.0	10	12
Water-12	83.3	81.3	169.7	97.0	10	15
Mean	**79.69**	**76.737**	**153.137**	**115.49**	**10.24**	**13.485**
LSD (0.05)	**6.68**		**3.84**		**0.468**	
CV %	**12.3621**		**2.39**		**3.3006**	

LSD, Least Significant Difference. CV, Coefficient of Variation.

Beneficial effect of treatment method on seed germination is indicated by three different indices; final germination percentage (FGP), mean germination time (MGT) and germination index (GI) (Kader, 2005). FGP indicates the percentage of germinated seeds and does not consider speed and uniformity of germination. FGP was highest for urea treated samples for both Manakamana-3 and Nutan-IL60 followed by urine treated plots. It was highly suppressed in salt and MOP treated plots (Table 3). Another index MGT is a measure of time taken for lot to germinate, but it fails to consider uniformity and germination percentage. Lower MGT indicates better effect. MGT was lowest in lots soaked in urea and urine for 24

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hours while, it was highest in lots soaked in water, salt and MOP solution for 12 or 24 hours in Nutan-IL60. However, MGT in Manakamana-3 was lower in water-soaked seed lot. Third parameter, GI is the most comprehensive measurement which considers germination percentage including speed and uniformity of germination. Higher GI indicated beneficial effect. In Manakamana-3, seed lot soaked in water and nitrogen source (urea and urine) produced better performance while salt and MOP treated lot were comparable to control. However, Nutan-IL60 showed better response specifically to treatments with nitrogen source (urea and urine).

Growth Parameters

Shoot and root length

Significant treatment effect was observed for shoot and root length of two maize varieties. Shoot and root were longest in urea and urine priming for both varieties (Figure 2 & 3). The root and shoot length of Nutan-IL60 primed in MOP, salt and water was statistically at par with control. While, all seed priming treatments significantly increased the shoot length of Manakamana-3 as compared to control but, only urine-based priming was effective to increase the root length (Figure 3). In general, urea and urine based seed priming showed some beneficial effects in terms of root and shoot length. This is mainly attributed to the accelerated metabolism occurring in primed seeds, which increases the imbibition speed as compared to un-primed seeds (control) in sorghum as reported by Tian *et al.* (2014).

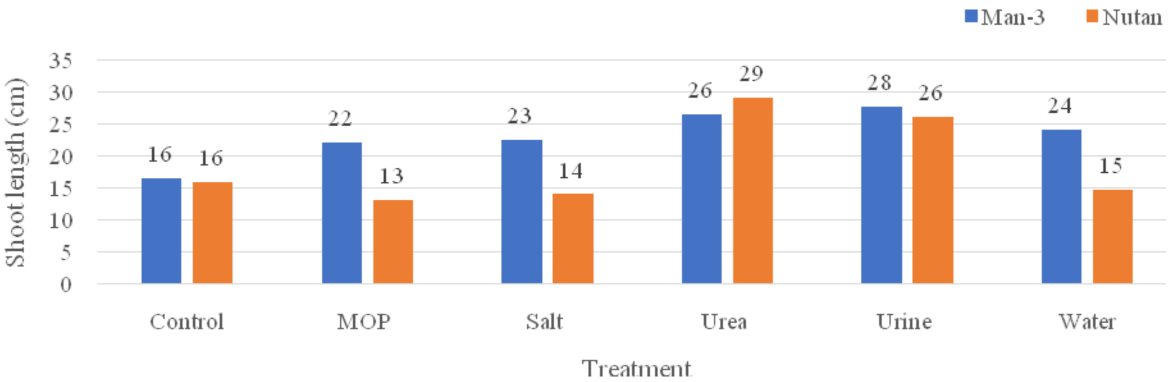

Figure 2: Effect of seed priming on shoot length of two maize varieties: Nutan-IL60 and Manakamana-3

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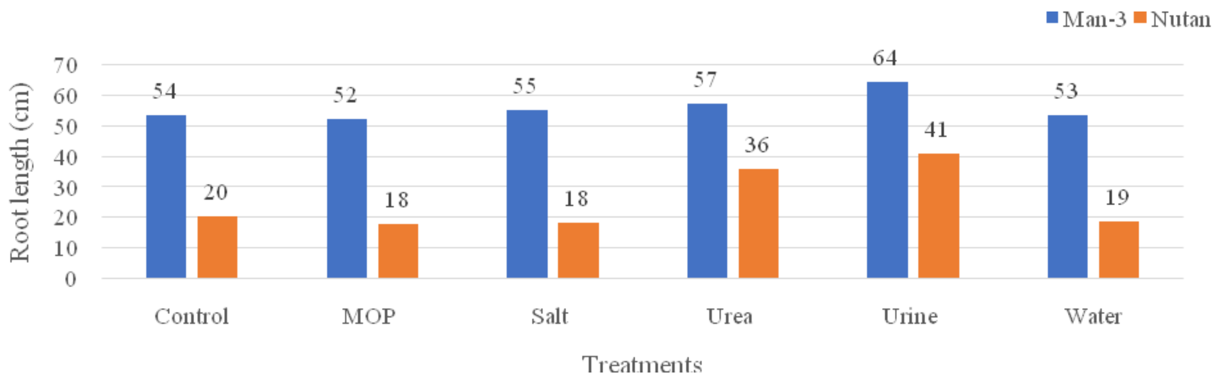

Figure 3: Effect of seed priming on root length of two maize varieties: Nutan-IL60 and Manakamana-3

Root dry weight

Root dry weight was significantly high under urea and urine based priming. The highest root weight was recorded from urea primed plot in Manakamana-3 and from urine primed plot in Nutan-IL60 (Figure 4). However, other treatments were comparable to control.

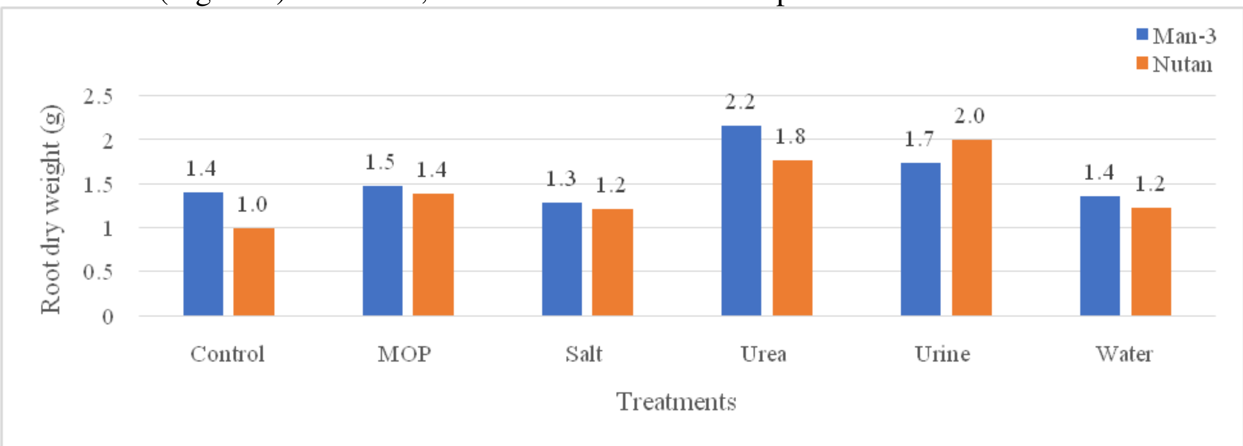

Figure 4: Effect of seed priming on dry root weight of two maize varieties: Nutan-IL60 and Manakamana-3

Osmo-priming is reported to increase the germination activities by increasing the activity of enzymatic antioxidants, superoxide dismutase (SOD) and peroxidase (POD) (Liu *et al.*, 2002), which helps in utilization of protein and carbohydrate during germination process (Kiran *et al.*, 2012). Traits like higher root length, dry root weight, and dry biomass facilitates a higher chance of stress tolerance and better yield performance. Horne, Ross and Hughes (1992) reported that maize genotypes with low root dry weight are less tolerant to drought stress. In our experiment, dry root weight is highest in nitrogen-based priming suggesting that these priming treatments enhance drought tolerance in both OPV and hybrid.

CONCLUSION

Seed priming is a simple technique for improving seed germination parameters and early seedling growth, which reduces the risk of poor crop establishment under rainfed condition. The results of this study showed that urine and urea based priming has a positive effect on

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germination and growth of two maize varieties, Manakamana-3 and Nutan-IL60. Priming with nitrogen-containing reagent showed significantly higher germination percent, mean germination index, seedling vigour, shoot length, root length and dry weight along with reduction in mean germination time. So, it may be concluded that varieties primed with nitrogen containing reagent will have a better chance of survival and growth.

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Authors' contributions

Avinash Shrestha	Trial Conduction, Data Recording, Data Analysis, Manuscript writing
Shreena Pradhan	Trial conduction, Data recording, Manuscript write-up
Jeny Shrestha	Manuscript write-up
Mahesh Subedi	Manuscript write-up and Data analysis

Conflict of interest
The authors declare no conflicts of interest regarding publication of this manuscript.

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